

MATING BEHAVIOR IN TWO TIGER BEETLES, *CICINDELA DORSALIS* AND *C. PURITANA* (COLEOPTERA: CICINDELIDAE)¹

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ABSTRACT: The copulatory behaviors of two species of tiger beetles were quantified. Mean duration of a complete copulatory sequence was much shorter for *Cicindela d. dorsalis* Say (6.1 min) and for *C. d. media* LeConte (6.2 min) than for *C. puritana* G. Horn (39 min). *Cicindela puritana* also had more pumping of the aedeagus and more reinsertions per pairing than *C. dorsalis*. Furthermore, the copulatory sequence of *C. puritana* differed from that documented from other cicindelids by exhibiting a large number of shallow pumps or movements of the aedeagus without deep insertion. Copulation in the species studied involved only partial withdrawal of the aedeagus and an absence of three clearly defined phases as described for *Pseudoxychila tarsalis* Bates. These differences in copulatory behavior among species and genera of tiger beetles may have important functional and phylogenetic significance.

Relative to many aspects of tiger beetle biology, little has been published about mating, particularly the quantification of copulatory behaviors and the variations among species. Willis (1967) reviewed the earlier literature on mating behavior (Mitchell 1902, Moore 1906, Shelford 1908, Goldsmith 1916) and observed mating in four species. He suggested the following mating behavior was typical of *Cicindela* species: 1. the male pursues the female in short runs until he overtakes her; 2. the male then uses his mandibles to grasp her on the mesepisternal coupling sulcus between the thorax and abdomen; 3. females often resist this behavior by shaking, turning, or rolling over; 4. the male then inserts the aedeagus one or several times within a several minute period; 5. then the male withdraws and dismounts. In some species the male continues "contact guarding" with the mandibles attached to the female (Kraus and Lederhouse 1983).

Palmer (1976) identified a 3-phase copulatory sequence in *Pseudoxychila tarsalis* Bates. The first phase was a deep insertion of the aedeagus, the second a complete withdrawal of the aedeagus and the third phase a deep reinsertion. Several species of *Cicindela* observed by Freitag *et al.* (1980) exhibited similar behavior except that the aedeagus was only partially withdrawn in phase 2. Schincariol (1988) reported the lack of a distinct phase 3 in *C. limbalis* Klug and *C. splendida* Hentz. Palmer (1976) suggested and Schincariol and Freitag (1986) demonstrated that in phase 1 the flagellum of the aedeagus is inserted into the spermathecal duct, possibly to clear it for subsequent transfer of the spermatophore contents in phase 3. These specific male and female mating behaviors thus relate to functional aspects of mating and

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can have a significant influence on sperm transfer precedence and sexual selection strategies in tiger beetles.

These studies and our unpublished observations of mating in several tiger beetle species indicated there are variations in this 3-phase sequence in *Cicindela*. Our study reports on detailed observations and quantifications of the specific copulatory behaviors of two species of tiger beetles, *Cicindela dorsalis* (subspecies *C. d. dorsalis* Say and *C. d. media* LeConte) and *C. puritana* G. Horn.

MATERIALS AND METHODS

Adult tiger beetles used in these studies were collected (under a USFWS permit, PRT-697823) from the following sites in late June-early July, 1993: *C. d. dorsalis* from Picketts Harbor, Northampton, Co., VA; *C. dorsalis media* from Fishermans Island, Northampton Co., VA; *C. dorsalis media* from Fishermans Island, Northampton Co., VA; (permit from Eastern Shore of Virginia National Wildlife Refuge); and *C. puritana* from Calvert County, MD and from near Cromwell, Hartford Co., CT (permit from CT Department of Game and Wildlife). The *C. dorsalis* and *C. puritana* which survived to the end of the study were returned to the collection sites. The beetles were collected with an insect net and placed in individual vials and transported to the laboratory in a cooler with ice. In the laboratory the beetles were maintained in plastic chambers (18 x 8 x 11 cms) with 3-6 cms of habitat soil and a screened top. The soil was moistened daily with a squeeze bottle. Adult flour beetles (*Tribolium*) and small bits of ground beef were introduced daily as food. Each chamber contained six beetles of one species; all the same sex. Air temperature in the air-conditioned laboratory was maintained at 23-27° C. with a small thermostatically controlled space heater. Soil surface humidity in the chambers was kept high (above 85%) by evaporation from the moist soil surface. Observations of the mating sequence started by placing a pair of beetles onto damp paper toweling covering the bottom of a 2 x 4 x 5 cms plastic chamber. All behaviors were timed on a stopwatch and described on a tape recorder, starting with the male's first attempt at subduing the female. One or more complete mating sequences were observed and recorded for at least 15 pairs of each of the three taxa (see Table 1). Mean numbers and times of the specific copulatory behaviors in the three taxa were compared using Kruskal-Wallis Anova.

RESULTS

Mating in *C. d. dorsalis* began with the male moving quickly toward the female, then attempting to mount her and fix his mandibles into her coupling

sulcus (a pair of pits on the mesepisternum at the anterior end of the elytra). Mean time from mounting to fixing the mandibles onto the female was 0.57 min (range of 0.02-8.2 min). The female usually shook violently, twisted from side to side or rolled on her back to dislodge the male from amplexus, but was seldom successful (6 of 38 times) (Table 1). Almost immediately after mounting the female and securing his mandibles, the male inserted the aedeagus nearly completely into the female's genital capsule with the top of the male's abdomen almost touching that of the female. Usually the male partially withdrew his aedeagus and reinserted deeply one or several more times (mean of 2.4, range of 1-6 times). This deep insertion or "pumping" involved the male flexing the tip of his abdomen forward, and corresponds to phase 1 of mating sequence Palmer described. Mean duration of phase 1 was 0.94 min (range 0.3-2.8). This was followed by a partial withdrawal of the aedeagus until only the 1-1.5 mm terminal portion (1/3-1/4 of the total length) was inserted. This phase was most comparable to Palmer's phase 2 which involved complete withdrawal. Next, there occurred a series of shallow pumps (mean of 5.8, range of 0-14) which moved the aedeagus only 1-2 mm deeper. Mean duration of this shallow pumping period was 5.2 min (range of 0.8-13.7). This activity most closely corresponds to Palmer's phase 3.

In 94% of the trials, the female attempted to dislodge the male (as described above) when the aedeagus was partially withdrawn. In these cases the male inserted the aedeagus deeply for several seconds, using his forelegs, middle legs or both to subdue the female. After the last shallow pump, the aedeagus was completely withdrawn and the pairing ended with the male retracting his mandibles from the coupling sulcus of the female. Mean duration of the total copulatory sequence, phases 1-3, was 6.12 min. In five of 30 trials, the male did not release his mandibles but continued contact guarding (Kraus and Lederhouse 1983) of the female for a variable length of time. In 9 of 15 cases the male attempted to remate with the female within the 60 min observation period (see below), and in 6 of these cases was successful in repeating the complete sequence. The mean number of complete mating sequences (insertions) within the 1 hour observation time was 2.0 (range 1-4) and the mean duration of pairing (insertion time and contact guarding) was 26.2 minutes.

The mating behavior of *C. d. media* was nearly identical to that of *C. d. dorsalis*, and with only slight differences in most of the parameters (Table 1). *C. d. media* had significantly more ($p < .01$) copulatory sequences per 60 minutes of observation (mean of 3.5) than *C. d. dorsalis* (mean of 2.0).

Mating behavior in *Cicindela puritana* differed from *C. dorsalis* in several significant ways. Females of *C. puritana* usually did not resist the initial

contact by the male and were thus immediately mounted (mount to subdue or mandible insertion interval averaged .02 min). Males also suspended themselves over the female using the middle and hind legs for support instead of resting their abdomens on the female's dorsum. Insertion of the aedeagus usually followed immediately after mounting, but in 3 of 14 cases the male remained in amplexus for several minutes before inserting. The initial insertion of the aedeagus of *C. puritana* was shallow, only 1-2 mm. This was followed by a series of shallow pumps in which the male pivoted at the abdominal-thoracic suture so that his abdomen moved ventral and anterior to its original position. This movement was very different from that in *C. d. dorsalis* which flexed only the abdominal tip, and resulted in only a 1-1.5 mm deeper insertion than when partially withdrawn. The mean number of pumps (all shallow) by *C. puritana* during the complete mating period was significantly greater than for *C. dorsalis* (mean of 16.8 versus 7.4 [$p < .05$]). Also, the interval between pumps was significantly shorter and the duration of the complete copulatory sequence significantly greater in *C. puritana* (38.9 min).

Postcopulatory contact guarding occurred in 14 of 16 cases, for a mean of 3.0 minutes. In several cases the male reinserted and repeated the mating sequence during this period (mean of 1.7 matings). Because of the longer copulatory time and the contact guarding there was little time for repeat mating sequences during the observation period (mean of 1.5 matings per 60 min). Females often mildly resisted the males reinsertion attempt by rapidly rubbing their middle legs against the male's and rolling back and forth. These attempts usually lasted only a few seconds and rarely resulted in the male releasing his grasp and dismounting. Immediately after withdrawal the male rubbed his forelegs against the female's antennae, something not seen in *C. dorsalis*.

DISCUSSION

Studies of other cicindelids have not included sufficient details of the various copulatory behaviors for an adequate comparison with the species we studied. However, we can make a comparison with the 3-phase mating sequence described in *Pseudoxychila tarsalis* (Palmer 1976). *Pseudoxychila tarsalis* and all species of *Cicindela* studied exhibit the initial grasping behavior and insertion of the aedeagus, although in *C. puritana* there is no deep insertion as seen in the other species. Unlike the complete withdrawal in *P. tarsalis*, all *Cicindela* then withdraw the aedeagus only partially. This most closely corresponds to Palmer's phase 2, but there is nothing comparable to Palmer's phase 3 (the deep reinsertion) in the mating of these *Cicindela*. The pumping and flexing which immediately follows the partial withdrawal may correspond to phase 3. Freitag *et al.* (1980) observed in their study of five

species that there were several deep insertions prior to final withdrawal which they suggested were phase 3. Schincariol (1988) indicated there was no phase 3 in *C. limbalis*, but he did note several pumps during the partial withdrawal phase, which seem to closely match what Freitag observed. Copulation in *C. puritana* was different from all other species described in having only shallow insertion and pumps.

These differences in mating behavior, although minor, may indicate important functional differences or have significant phylogenetic implications. For example, if the initial deep pumps or insertions serve to clear sperm from the spermathecal duct as demonstrated by Freitag *et al.* (1980), the absence of this in *C. puritana* may mean clearing does not occur or may be done in a different manner. The contact guarding in *C. puritana* could be an alternative mechanism of sperm precedence, insuring that the male which is able to mate with and stay complexed with a female will fertilize her eggs. Kraus and Lederhouse (1983) suggested that contact guarding in *C. marutha*, a species very closely related to *C. puritana* and apparently with similar mating behavior, may be associated with the high level of intrasexual competition among males. Both of these species occur at high densities and male-to-male interactions are likely to be frequent.

It seems apparent that since *Pseudoxychila* is regarded as ancestral to the *Cicindela*, the three phase copulatory behavior which it exhibits may be a precursor to the modified mating pattern seen in species of *Cicindela* and/or related to morphological differences in genitalia. This is consistent with the suggestion of Freitag *et al.* (1980) that *Cicindela* has evolved the coupling sulcus (absent in *Pseudoxychila*) as a mechanism to insure successful mating in the more highly active, diurnal tiger beetles. In a similar manner, a more abbreviated mating sequence may have also been selected for in *Cicindela*.

It is hoped that these observations and interpretations will stimulate interest in this important and somewhat ignored aspect of tiger beetle biology. We agree with Schincariol (1988) that a thorough comparative study of mating behavior in tiger beetles is needed.

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Table 1. Mating parameters for *C.d.dorsalis*, *C.d.media*, and *C.puritana*. Based on 60-90 minute observations for each pair.

Mating parameter	<i>C.d.dorsalis</i>			<i>C.d.media</i>			<i>C.puritana</i>			p value
	n	x	sd	n	x	sd	n	x	sd	
Mount to subdue interval	23	0.57	1.65	16	0.25	0.06	17	0.02	0.06	<0.001
Total number of mounts per 60 min.	16	2.5	1.87	7	4.29	0.91	14	1.5	0.91	>0.05
Pairing time per copulatory sequence	22	26.24	31.66	17	28.34	39.05	19	38.99	37.36	>0.05
Total number of copulations per 60 min.	15	2.0	0.97	6	3.5	0.28	12	1.08	0.28	<0.01
Duration of complete copulatory sequence	19	6.12	5.14	18	6.21	4.72	19	38.99	8.13	<0.01
Interval between copulatory sequences	19	16.38	25.64	22	10.87	15.1	5	29.66	24.13	>0.05
Number of reinsertions per copulatory sequence	26	0.15	0.46	18	0.11	0.46	11	1.73	0.75	<0.001
Interval between reinsertions per copulatory sequence	4	24.8	14.4	2	36.29	19.44	19	7.04	19.44	>0.05
Total number of pumps including all phases (<i>C.d.m.</i> , <i>C.d.d.</i> , <i>C.p.</i>)	18	7.39	3.51	19	7.68	5.78	25	16.8	13.36	<0.05
Duration of Phase 1 (<i>C.d.m.</i> & <i>C.d.d.</i>)	19	0.94	0.63	18	1.15	1.09	—	—	—	>0.05
Total number of deep pumps (<i>C.d.m.</i> & <i>C.d.d.</i>) (Phase 1)	17	2.41	1.46	17	3.35	2.83	—	—	—	>0.05
Duration of Phase 2 (<i>C.d.m.</i> & <i>C.d.d.</i>)	19	5.20	4.99	18	5.08	4.44	—	—	—	>0.05
Total number of shallow pumps (<i>C.d.m.</i> & <i>C.d.d.</i>) (Phase 2)	16	5.75	3.7	18	4.94	3.89	—	—	—	>0.05
Interval between deep pumps (or pumps for <i>C.p.</i>)	23	0.16	0.05	37	0.17	0.07	100	0.13	0.07	<0.05
Interval between shallow pumps (or pumps for <i>C.p.</i>)	69	0.23	0.08	58	0.23	0.06	104	0.13	0.07	<0.001

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INSECTS. LIFE CYCLES AND THE SEASONS. J. Brackenbury. 1994. Blanford, London. Distrib. in U.S. Sterling Publish. Co., 387 Park Ave. South, NY, NY 10016-8810. 9 x 11 format. 192 pp. \$24.95 Hdbk.

This book chronicles some of the intriguing relationships between insects and their life cycles, attuned to the four seasons associated with the temperate climates. With introductory texts for easy, popular reading and with expanded legends accompanying fine photographs, all in color, this book presents a striking pictorial account of these natural sequences. Primarily directed to amateur entomologists and naturalists.

INSECTS IN FLIGHT. J. Brackenbury. 1992. Blanford, London. Distrib. in U.S. Sterling Publish. Co., 387 Park Ave. South, NY, NY 10016-8810. 9 x 11 format. 192 pp. \$35.00 Hdbk.

This book shows how insects take an entirely different approach from that of birds in the design of the flight machine. With easily readable texts that both fascinate and entertain and with expanded legends accompanying the many fine photographs, all in color, this book unveils the world of insect flight for the amateur entomologist and naturalist.